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Research Article

Impact of Interleukin-6 Serum Levels and rs1800795 Promoter Polymorphism on Disease Activity and Tumor Necrosis Factor Inhibitor Efficacy in Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a systemic inflammatory disease. Gene polymorphisms of cytokines play a pivotal role in their pathogenesis and are able to modulate susceptibility and responsiveness for RA treatment. ***Objective***: To investigate the serum expression of the rs1800795 variant of interleukin-6 (IL-6) and its association with disease severity and tumor necrosis factor (TNF)-α inhibitor response in RA. ***Methods***: A case-control study was conducted, including 100 patients with RA and 100 healthy controls. Serum levels of IL-6 were measured. Genotyping of the IL-6 rs1800795 polymorphism was performed using TaqMan real-time PCR. Disease activity was assessed using the Clinical Disease Activity Index (CDAI), and treatment response was evaluated after six months of TNF inhibitor therapy. ***Results***: Rheumatoid arthritis patients had significantly greater levels of IL-6 compared to the control group (p<0.001), with the diagnostic accuracy area under the curve (AUC) equal to 0.999. The mutant C allele of rs1800795 was significantly associated with increased RA risk (OR=1.70, p=0.022) and poor response to TNF inhibitors (OR=2.26, p=0.009). Higher IL-6 levels and the presence of the CC genotype were correlated with higher disease activity and lower treatment responsiveness (p<0.001). ***Conclusions***: Elevated serum IL-6 levels and carriage of the IL-6 rs1800795 C allele are associated with susceptibility to RA, disease severity, and reduced response to TNF inhibitors. These findings prove the therapeutic potential of the IL-6 biomarker for patient stratification and optimized therapeutic strategies in RA therapy.

Keywords: Interleukin-6, Rheumatoid arthritis, rs1800795 polymorphism, Tumor necrosis factor inhibitors.

تأثير مستويات مصل إنترلوكين -6 وتعدد أشكال المحفز rs1800795 على نشاط المرض وفعالية مثبط عامل نخر الورم في التهاب المفاصل الرثوي

الخلاصة

الخلفية: التهاب المفاصل الرثوي هو مرض التهابي جهازي. تلعب تعددات أشكال جينات السيتوكينات دورًا محوريًا في أمراضيته، ويمكنها تعديل القابلية للإصابة والاستجابة لعلاج المرض. **الهدف**: دراسة تعبير المتغير الجينيrs1800795 من الإنترلوكين-6 في المصل، وعلاقته بشدة المرض والاستجابة لمثبطات عامل نخر الورم (TNF-α) في التهاب المفاصل الرثوي. **الطرائق**: أجريت دراسة حالة-شاهد شملت 100 مريض مصاب بالتهاب المفاصل الرثوي و100 شخص سليم كضابط. تم قياس مستويات انترلوكين-6 في المصل، وتم تحديد النمط الجيني لتعدد الأشكالrs1800795 الخاصة بهذا السيتوكين باستخدام تقنية تفاعل البوليميراز المتسلسل الكمي (real-time PCR) . تم تقييم نشاط المرض باستخدام مؤشر نشاط المرض السريري(CDAI) ، وتم تقييم الاستجابة للعلاج بعد ستة أشهر من استخدام مثبطات عامل نخر الورم. **النتائج**: أظهر مرضى التهاب المفاصل الرثوي مستويات أعلى من انترلوكين-6 في المصل مقارنةً بمجموعة الضبط (p<0.001)، مع دقة تشخيصية (AUC) بلغت 0.999. كان وجود الأليل المتحور C من1800795 rs مرتبطًا بشكل ملحوظ بزيادة خطر الإصابة بالمرض (OR=1.7, p=0.022) وضعف الاستجابة لمثبطات عامل نخر الورم (OR=2.26, p=0.009). كما ارتبطت المستويات الأعلى من انترلوكين-6 ووجود النمط الجيني CC بنشاط مرضي أعلى واستجابة علاجية أقل (p<0.001). **الاستنتاجات**: ترتبط المستويات المرتفعة من انترلوكين-6 في المصل وحمل أليل C من1800795 rs في انترلوكين-6 بزيادة القابلية للإصابة بالتهاب المفاصل الرثوي، وشدة المرض، وانخفاض الاستجابة لمثبطات عامل نخر الورم. تدعم هذه النتائج الدور العلاجي المحتمل للمؤشر الحيوي انترلوكين-6 في تصنيف المرضى واستراتيجيات العلاج المثلى في التهاب المفاصل الرثوي.

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INTRODUCTION

Rheumatoid arthritis is a long-standing inflammatory disorder. The etiopathogenesis of RA is multifactorial, with genetic predisposition, environmental elements, and deranged immune processes involved. One of the most significant pro-inflammatory cytokines of RA pathogenesis is IL-6, a pleiotropic cytokine that orchestrates the inflammatory cascade. In RA, chronic

stimulation of the IL-6/JAK/STAT3 pathway sustains inflammation, synovial hyperplasia, and joint destruction. IL-6 also promotes the differentiation of Th17 cells and inhibits the activity of regulatory T cells, which results in chronic irritation and autoimmunity [1]. Interleukin-6 gene promoter region polymorphism rs1800795 (G>C) is associated with altered IL-6 expression. Various studies have confirmed that the C allele was connected with higher levels of serum IL-6 as well as more susceptibility to RA in particular groups [2]. However, findings of this association have been replicated within different populations in a variable way, suggesting that genetic factors and gene–environment interaction can modulate observed effects [3]. Even with the improvement in RA treatment, 30% of RA patients still have suboptimal or no clinical response to TNF inhibitors, making it essential to identify predictive biomarkers for therapeutic decision-making [4]. IL-6, based on its key role in RA pathogenesis and inflammation, has been suggested as a candidate biomarker for disease activity and treatment response [5]. Therefore, the present study aims to compare serum concentration and the rs1800795 gene polymorphism of IL-6 in RA patients with healthy controls to analyze their association with disease activity and response to TNF inhibitors, which aids in the orientation of more specific, individualized treatment approaches, a path highly pertinent in the precision medicine era.

METHODS

Study design and setting

This case-control study was conducted on 200 subjects in all, of which 100 were RA patients and 100 as healthy control group who were matched for age and sex. RA patients were recruited from the Baghdad Teaching Hospital and Al-Yarmouk Teaching Hospital Rheumatology Units from March to November 2023. Diagnosis was made by an experienced rheumatologist according to the European Alliance of Associations for Rheumatology (EULAR) 2022 update [6] and the American College of Rheumatology (ACR) 2021 [7] classification criteria. The RA patient group was also split into two groups based on response to TNF inhibitors (infliximab or etanercept) after 6 months of treatment: Responders (n=50): CDAI ≤ 10; non-responders (n=50): CDAI > 10.

Inclusion and exclusion criteria

The inclusion criteria were adult patients (≥18 years) with definite RA and biological therapy with TNF inhibitors for 6 months. The exclusion criteria were pregnancy, malignancy, and other autoimmune or chronic systemic diseases.

Sample selection and outcome measurements

Venous blood samples (2.0 mL) were collected from each participant. Serum IL-6 levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, USA) according to the manufacturer's protocol. Genomic DNA was extracted from whole blood using the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA). DNA concentration and purity were quantified by a Quantus™ Fluorometer. Allelic discrimination of IL-6 rs1800795 polymorphism was carried out by TaqMan probe-based real-time polymerase chain reaction (quantitative PCR) on the Mic quantitative PCR cycler (Bio Molecular Systems, Australia). The primers and probes used were as follows, as stated in Table 1.

Table 1: Primer and probe sequences used for IL-6 rs1800795 genotyping

Gene	Primer/probe Name	Sequence 5`- 3`
IL-6	rs1800795-F	TGACGACCTAAGCTGCACTT
	rs1800795-R	GATTGTGCAATGTGACGTCCTT
	rs1800795-P/G	FAM-TTGTGTCTTGCCATGCT
	rs1800795-P/C	HEX-TGTGTCTTGCGATGCTA

The PCR program consisted of a first denaturation at 95℃ for 5 minutes and then 40 denaturation at 95℃ for 20 seconds, annealing at 60℃ for 20 seconds, and extension at 72℃ for 20 seconds.

Ethical consideration

Informed consent was gained from all participants, and the Institutional Review Board of Al-Nahrain University, College of Medicine (Approval No. M.M.M/24, Date: 22/2/2023), as per the Declaration of Helsinki, approved the study.

Statistical analysis

Statistical analysis was carried out using SPSS software version 25.0 (IBM Corp., USA). A two-tailed p-value of 0.05 was considered significant. Parametric data were analyzed using the independent t-test, non-parametric data with the Mann–Whitney U test, and categorical variables with the chi-square test. Binary logistic regression estimated odds ratios (ORs) and 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curve analysis evaluated IL-6 diagnostic performance.

RESULTS

No significant differences were recorded between RA patients and the control group on age (mean ± SD: 51.970 ± 10.506 years among patients *vs*. 50.480 ± 10.968 years in controls, p= 0.328) or sex ratio (80% females in patients *vs*. 75% in controls, p= 0.397).

However, RA patients exhibited significantly elevated rates of a positive family history (34% vs. 0%, $p < 0.001$), smoking history (30% vs. 13%, $p = 0.003$), and body mass index (BMI) (28.889 ± 5.674 vs. 25.271 ± 4.325 kg/m², $p < 0.001$). In addition, overweight and obesity prevalence were higher in the RA group (33% and 39%) than in the controls (19% and 27%, respectively; $p = 0.001$). The median serum concentration of IL-6 was significantly higher in RA patients (6.197 pg/mL) compared to the control group (0.359 pg/mL, $p < 0.001$). ROC curve analysis demonstrated that IL-6 had excellent discriminative ability, with an AUC of 0.999 (95% CI: 0.998–1.000). At a cut-off value of 0.278 pg/mL, sensitivity was 98% and specificity was 100%. The *p*-value was less than 0.001, indicating strong statistical significance (Figure 1).

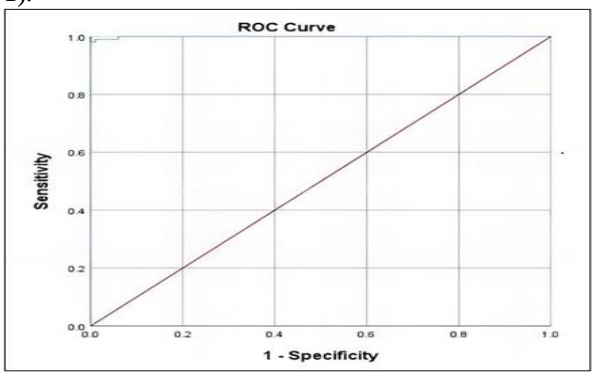

Figure 1: Receiver operating characteristic curve for IL-6 in discrimination between patients with RA and controls.

The distribution of IL-6 rs1800795 genotypes and alleles in patients and controls is summarized in Tables 2 and 3.

Table 2: Distribution of different genotypes and alleles of the polymorphism (rs1800795) of IL-6 in cases and the control group

rs1800795 (G>C)	Patients (n=100)	Control (n=100)	OR (95%CI)	*p-value*
Genotypes				
GG	50(50)	63(63)	0.58 (0.33-1.03)	0.064
GC	39(39)	33(33)	1.29 (0.72-2.31)	0.377
CC	11(11)	4(4)	2.96 (0.91-9.65)	0.071
HWE*	0.423	0.901		
Dominant model				
(GG+GC)	89(89)	96(96)	0.33 (0.10-1.09)	0.071
(CC)	11(11)	4(4)	2.96 (0.91-9.65)	
Recessive model				
(GG)	50(50)	63(63)	0.58 (0.33-1.03)	0.064
(GC+CC)	50(50)	37(37)	1.70 (0.96-2.99)	
Alleles				
G	139(69.5)	159(79.5)	0.58 (0.37-0.92)	0.022
C	61(30.5)	41(20.5)	1.70 (1.07-2.68)	

Values are presented as frequency and percentage.* HWE= Hardy–Weinberg equilibrium.

Table 3: Frequency of different genotypes and alleles of IL-6 polymorphism in responder and non-responder patients

Rs1800795 (G>C)	Non-responder (n=50)	Responder (n=50)	OR (95%CI)	*p-value*
Genotypes				
GG	21(42)	29(58)	0.52 (0.23-1.16)	0.111
GC	19(38)	20(40)	0.91 (0.41-2.05)	0.837
CC	10(20)	1(2)	12.25 (1.50-99.80)	0.019
Dominant model				
(GG+GC)	40(80)	49(98)	0.08 (0.01-0.66)	0.019
(CC)	10(20)	1(2)	12.25 (1.50-99.80)	
Recessive model				
(GG)	21(42)	29(58)	0.52 (0.23-1.16)	0.111
(GC+CC)	29(58)	21(42)	1.90 (0.86-4.21)	
Alleles				
G	61(61)	78(78)	0.44 (0.23-0.82)	0.009
C	39(39)	22(22)	2.26 (1.21-4.21)	

Values are expressed as frequency and percentage.

The amplification plot (Figure 2) demonstrates clear allelic discrimination using the TaqMan assay for rs1800795 genotyping. While the wild-type homozygous genotype (GG) was more common in controls, the mutant allele (C) showed a significantly higher frequency among patients (30.5% vs 20.5%; OR = 1.70, 95% CI = 1.07–2.68, *p*= 0.022).

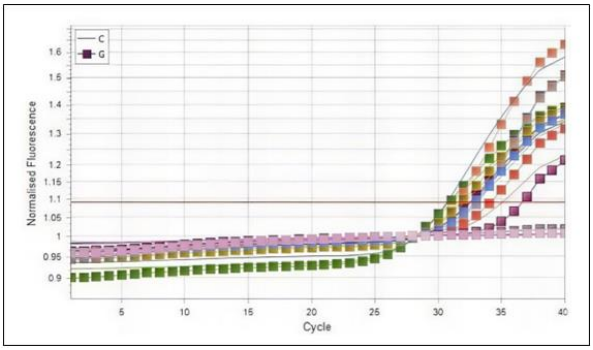

Figure 2: Amplification plot for IL-6 (rs1800795).

Genotype frequencies in both groups were in Hardy–Weinberg equilibrium (HWE). No significant differences were observed for the heterozygous (GC), homozygous mutant (CC), or dominant/recessive genetic models (Table 2). In addition, there was no statistically significant difference between responders and non-responders regarding age, sex, family history of RA, smoking, or BMI. Non-responders' mean age was slightly higher (52.420±10.250 years) compared with responders (51.520±10.842 years). The majority of responders and non-responders were within the age group of 40–60 years. Males dominated the responders (26%) compared to non-responders (14%), but this was not statistically significant. Similarly, the family

history of RA and smoking also were not statistically associated with the outcome of treatment. Though in non-responders a greater mean BMI as well as a greater frequency of obesity (48% vs. 30%) was observed, these figures were not found at the level of statistical significance. Methotrexate (MTX) use was higher in non-responders (76%) than responders (60%), although this was not significant (p = 0.132). For use of TNF inhibitors, infliximab use was significantly higher in responders (50%) compared with non-responders (28%; p = 0.039), whereas etanercept was used more by non-responders (72% vs. 50%). Branded biologics analysis did not reveal any statistically significant variations across the particular use of Remicade, Ixifi, or Remsima despite the fact that Enbrel (etanercept) was used more among the non-responders (72%). Non-responders exhibited extremely high levels of IL-6, with a median of 9.517 pg/mL as opposed to responders, who presented with 5.211 pg/mL (p < 0.001), suggesting an inverse relationship between the IL-6 inflammatory biomarker and reduced response to TNF inhibitor therapy. The distribution of IL-6 rs1800795 genotypes and alleles amongst responders and non-responders is provided in Table three. The homozygous mutant genotype (CC) and the mutant C allele have been extensively more common in non-responders, indicating an affiliation with an extended threat of non-responsiveness. In contrast, genotypes containing the wild-type G allele (GG+GC) were significantly more common among responders. Other genotype comparisons showed no statistically significant differences. Serum IL-6 levels were significantly associated with disease activity, as patients with higher CDAI scores exhibited higher median IL-6 concentrations, while those in remission had the lowest levels (Table 4).

Table 4: CDAI association with IL-6 serum levels

CDAI score (n=100)	IL-6 (pg/mL) Median (min–max)
Remission	3.419 (2.161-4.032)
Low	5.229 (3.129-10.129)
Moderate	6.370 (0.935-48.419)
High	7.903 (3.032-67.838)
p-value	0.002

Serum IL-6 concentrations tended to be higher in patients with the mutant homozygous (CC) genotype compared to other genotypes, but this difference was not statistically significant (Table 5).

Table 5: Serum IL-6 Levels According to rs1800795 Genotypes of the IL-6 Gene

SNP gene	Genotypes	IL-6 (pg/mL) Median (Min–Max)	*p-value*
IL-6 rs1800795 (G>C)	GG	5.967 (0.935-31.741)	0.929
	GC	6.197 (2.161-67.838)	
	CC	6.806 (3.129-21.123)	

The distribution of IL-6 (rs1800795) genotypes by CDAI disease activity levels is shown in Table 6. There was a significant association between genotype and disease severity (p< 0.001), with the CC genotype predominating among patients with the highest disease activity, while the GG genotype was most frequent in those in remission. There was a significant variation in CDAI scores among patients treated with different infliximab and etanercept brands (p= 0.037).

Table 6: Distribution of IL-6 (rs1800795) Genotypes by CDAI Disease Activity Levels and Statistical Association

SNP genes	Genotypes	CDAI score (n=100)				*p-value*
		Remission	Low	Moderate	High	
IL-6 rs1800795 (G>C)	GG	4(8)	19(38)	15(30)	12 (24)	<0.001
	GC	0(0.0)	2(5.13)	14(35.9)	23(58.97)	
	CC	0(0.0)	0(0.0)	1(9.09)	10(90.91)	

Values are expressed as frequency and percentage.

Remicade was most commonly associated with low disease activity, while Ixifi and Remsima were distributed between moderate and high activity. Enbrel showed the highest proportion of patients with high disease activity. The detailed distribution is presented in Table 7.

Table 7: Distribution of CDAI Scores based on TNF Inhibitor Brands (Infliximab and Etanercept)

Trademarks of Infliximab and Etanercept	CDAI score (n=100)				Total	*p*-value
	Remission	Low	Moderate	High		
Remicade (Infliximab)	0(0.0)	5(71.43)	0(0.0)	2(28.57)	7(100)	0.037
Ixifi (Infliximab)	1(9.09)	2(18.19)	4(36.36)	4(36.36)	11(100)	
Remsima (Infliximab)	0(0.0)	4(19.05)	10(47.62)	7(33.33)	21(100)	
Enbrel (Etanercept)	3(4.92)	10(16.39)	16(26.23)	32(52.46)	61(100)	

Values are expressed as frequency and percentage.

DISCUSSION

The demographic profile of the current research reported the mean age at time of diagnosis among RA patients to be 51.970 ± 10.506 years, which was most widespread within the range of 40 to 60 years. The increased incidence of RA in middle-aged individuals, particularly among women, has been predominantly noted. Vulnerability to RA with increasing age has been contributed by a range of immunological and environmental conditions, including immune senescence, thymic involution, psychological stress, and susceptibility to extrinsic risk factors like smoking, which may all contribute to the triggering of autoreactive lymphocytes involved in RA pathogenesis [8]. These results confirm the significance of age as a determining factor in RA onset and progression. In this current study, RA was significantly more prevalent among females (80%) than in males (20%), in accordance with previous research [9]. The sex disparity is due to a polyfactorial interaction between hormonal, genetic, and environmental influences. Estrogen modulates immune responses and cytokine production, and X-connected immune-regulatory genes might also beautify susceptibility. Moreover, environmental elements like smoking similarly augment RA threat, especially amongst genetically predisposed girls [10]. There became a big association between family records and accelerated risk of RA. This is in keeping with preceding findings that first-degree family (FDRs) of patients with RA have a two- to fourfold higher danger due to shared genetic and environmental determinants [11]. The present study confirms that smoking significantly increases the risk and severity of RA, as found in earlier studies [12]. Although the mechanisms are unknown, cigarette smoke has been shown to induce pro-inflammatory cytokines—such as IL-1α, IL-1β, IL-6, IL-8, and TNF-α—in fibroblast-like synoviocytes (FLS) of RA patients. These cytokines, particularly IL-1 and TNF-α, are worried about RA pathogenesis through promoting continual joint irritation [13]. Here, we tested the BMI of RA sufferers, which turned out to be 28.889 ± 5.674, suggesting a capacity relationship between weight problems and increased RA threat. This finding is in accordance with previous literature indicating that greater BMI is associated with RA development [14]. The pro-inflammatory role of adipose tissue, via adipokines secreted by adipocytes and macrophages, may be one reason for RA pathogenesis. These adipokines, including c-reactive protein (CRP), IL-6, TNF-α, and IL-1β, tend to be elevated in obese individuals and have been found at elevated levels during preclinical RA stages [15]. In line with previous research, the present research also identified very high levels of IL-6 in RA patients compared to healthy controls [16]. IL-6 plays a central role in pathogenesis in RA as it evokes acute-phase reactions and sustains chronic inflammation through the activation of lymphocytes and myeloid cells. The very high and labile levels detected signify chronic systemic inflammation that also contributes to disease severity and progression. In the present study, IL-6 was 98% sensitive and 100% specific in differentiating RA patients from controls, with greater diagnostic efficacy than has been previously noted. For comparison, Mohammed *et al*. had 92.5% sensitivity and 42.5% specificity [17]. These findings render IL-6 a highly sensitive biomarker of potential clinical value for the diagnosis of RA. Heterogeneity of study design, sample size, and cut-off values employed in the studies could be the possible reasons for biomarker sensitivity and specificity discordance. The results of this research showed an increased frequency of the C allele of the IL-6 rs1800795 SNP in RA patients versus controls, suggesting a potential role in susceptibility. In consonance with a recessive model, GC+CC genotypes were notably more frequent in patients and further defined the risk imparted by this polymorphism. These findings are consistent with the findings of previous studies in Asian and African populations, for example, in Chinese and Egyptians, where a significant association of the C allele with high risk of RA has been established [2]. Functionally, IL-6 promoter region polymorphism rs1800795 has been implicated in elevated levels of IL-6, promoting inflammation and joint damage [18]. However, reports from European and Latin American populations (Spanish, Turkish, and Polish) are contradictory and suggest that such an association may be modulated by ethnic genetic backgrounds, environmental exposures, or study design differences [3]. These differences constitute the foundations of the importance of gene-environmental interactions in the control of IL-6-mediated immune response and RA susceptibility. Our findings indicate the mean age for non-responders was 52.420 ± 10.250 years, and age response to therapy did not show a significant variation among various groups of age, namely the group 40–60 years old. This does not agree with Koczan *et al*.'s findings but cited an age-specific response toward TNF inhibitors [19]. Variations are thought to be on account of differential study population, population characteristics, or geographical setup. This study indicated that female RA patients were more frequently non-responders to TNF inhibitors than men. The reduced efficacy in ladies can be due to more than one factor, which includes hormonal impacts (e.g., estrogen-regulated immune reaction), sex-precise gene mutations, more disorder activity at baseline, and variations in TNF receptor expression [20]. Pharmacokinetic heterogeneity, compliance with remedy, and ache sensitivity will also be gambling a position. Psychological elements consisting of heightened melancholy in women might also affect immune reactions and remedy response. Collectively, those findings emphasize the multifactorial nature of

intercourse differences in RA reaction to remedy. The current research confirmed that there was no significant association between RA family history and response to anti-TNF treatment. While genetic predisposition to RA is linked with specific HLA-DRB1 alleles, they do not appear to directly influence the response to treatment [21]. The genetic shape of RA is multifactorial and encompasses many gene-environment interactions, and a family record isn't always a terrific predictor. Furthermore, disease length, initial interest, comorbidities, and environmental factors like smoking had been cautioned to play a greater role in TNF inhibitor reaction modulation [22]. These findings affirm that the RA response to remedy is complex and no longer genetically decided. Evidence from this study indicates that smoking has been repeatedly associated with a less desirable clinical response to TNF inhibitors in RA patients. Several studies have demonstrated that smokers or past smokers receive less effective treatment, increased disease activity, and more extra-articular manifestations [23]. Though Söderlin *et al*. could not prove definitive data that smoking cessation has an association with better treatment outcome, the destructive impact of smoking is clear [24]. Mechanistically, smoking augments citrullination of proteins, leading to higher levels of Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), both being adverse predictors [25]. Smoking also amplifies systemic inflammation through augmentation of TNF-α as well as other inflammatory cytokines capable of obscuring the therapeutic effect of TNF inhibitors. Further, smoking could alter drug pharmacokinetics by reduced absorption or increased clearance. Taken together, the findings indicate the negative impact of smoking on RA disease activity and drug response. The present study revealed that RA patients who were overweight responded less to TNF inhibitor treatment, as was previously predicted by meta-analyses and real-world data [26]. Drug pharmacokinetic modulation is characterized by obesity, increased fat mass, and raised adipokine levels of leptin stimulating the release of pro-inflammatory cytokines like TNF-α and IL-6 [27]. These pathways are involved in heightened inflammatory status and reduced therapeutic response. Notably, within class III obesity (BMI >40 kg/m²), patients also had poorer treatment survival and increased residual synovitis, even after clinical remission, emphasizing the contribution of obesity to treatment response and disease control. This study observed that there were more non-responders receiving concomitant MTX compared to responders, showing no discernible association of MTX usage with augmented TNF inhibitor response. This observation is in agreement with other reports, which demonstrated no significant effect of MTX co-therapy on RA outcomes in a large RA population [28]. The heterogeneity of MTX efficacy can be attributed to genetic polymorphisms, notably in HLA-DRB1 alleles [29], and also to variability in patient compliance [30] and treatment duration. These factors refer to the difficulty in optimizing combination therapy with MTX in RA. The research demonstrated a higher response to infliximab compared to etanercept in RA patients, contradicting findings by Zervou *et al*., which demonstrated the two drugs were equal [31]. Heterogeneity of drug response could be attributed to genetic polymorphisms that influence susceptibility to single TNF inhibitors in patients [32]. Moreover, serum drug concentration is also essential; low levels of etanercept have been associated with nonresponse [33]. At the mechanistic level, infliximab binds greater immune complexes, facilitating greater antigen-presenting cell uptake, while etanercept has lower immunogenicity and fewer foreign epitopes [34]. Such pharmacological differences augmented by patient-related factors can account for varied therapeutic effects. Findings from this research suggest that a higher percentage of non-responders were on etanercept (Enbrel), while infliximab-class medicines (Remicade, Ixifi, and Remsima) have been more frequently associated with response to remedy. These findings are in agreement with Aghdashi *et al*. [35]. Genetic polymorphisms in genes that affect TNF receptor function and immune regulation underline inter-individual variability in response to TNF inhibitors. Mechanistically, etanercept acts as a soluble receptor to capture TNF, while infliximab is a monoclonal antibody that directly neutralizes TNF, with possible augmented efficacy in patients with high inflammatory burden [36]. Fairly high non-responder serum levels of IL-6 in this study are consistent with reports that IL-6 level reduction is linked with better biologic Disease-Modifying Anti-Rheumatic Drug (bDMARD) therapy outcomes [37]. This suggests that other pathways, i.e., the IL-6/STAT3 pathway, are implicated in non-responder chronic inflammation that is still functional despite TNF blockade [38]. Moreover, TNF inhibition may be the reason for compensatory overexpression of IL-6 in a few patients [5]. High ranges of IL-6 have been implicated in treatment resistance, joint damage, and disease activity and are a predictor of a more competitive RA phenotype [39]. Genetic susceptibility will also be the muse for the partial effectiveness of TNF inhibitors with sustained IL-6 signaling. The outcome of the research indicated that RA patients with carriage of the IL-6 rs1800795 C allele (CC or GC genotype) were more likely to be non-responders to TNF inhibitors, and the GG genotype was predictive of effective therapeutic response. This result is supported by the findings of Dávila-Fajardo *et al*., who identified this polymorphism as a promising biomarker for response to anti-TNF therapy [40]. From a mechanistic perspective, the C allele is linked to increased expression of IL-6, which promotes inflammation via

the IL-6/JAK-STAT3 pathway regardless of TNF blockade [41]. Contrarily, GG carriers exhibit reduced IL-6 levels and less compensatory inflammation, i.e., a better response. Variability in studies may be caused by ethnic genetic heterogeneity, treatment regimens, and clinical or environmental confounders [42]. Overall, rs1800795 may be a valuable pharmacogenetic biomarker for the personalization of treatment in RA. This study displayed a significant positive association between IL-6 serum levels and CDAI scores in RA patients, where IL-6 levels increased with disease activity and decreased in patients who went into remission. These findings are consistent with other studies showing that IL-6 levels are associated with clinical disease activity in RA. IL-6 plays a role in the pathogenesis of RA by inducing osteoclast activation and resulting bone resorption and cartilage destruction and by inducing acute-phase reactants like CRP and hence perpetuating systemic inflammation [43]. In present study, RA patients with IL-6 rs1800795 CC genotype had the highest median serum IL-6 of all GC and GG genotypes. These results are supported by other research to show that the C allele increases IL-6 expression [44], although opposing evidence exists to link the GG genotype with increased IL-6 [45]. Mechanistically, C allele is associated with augmented nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) binding on the IL-6 promoter and elevated mRNA stability and thereby longer-term production of IL-6. It also has associations with higher release of cytokines by macrophages and monocytes [46]. Ethnic and environmental differences can impact on the functional consequence of rs1800795 to generate heterogeneity observed among studies [47]. This research demonstrated a strong association between IL-6 rs1800795 genotypes and RA severity according to CDAI, with CC correlated with the most severe disease condition and GG with milder conditions. . The findings validated those of Alhilali *et al*., which reported elevated IL-6 levels and disease activity in CC genotype carriers [3]. Mechanistically, the CC genotype increases the transcription of IL-6, which results in greater serum levels of IL-6 and increased inflammatory burden, supporting its pathogenesis in RA [48]. From the data at hand, it is evident that there is a high association between TNF inhibitor therapy and disease activity measured by CDAI, with infliximab agents (e.g., Remicade) having greater efficacy in lowering disease activity compared to etanercept (Enbrel), which was associated with a higher proportion of patients with high disease activity. These are pharmacodynamic, immunogenic, and molecular structural differences that have been proven between TNF inhibitors. Though they are able to blunt disease activity, remission is not typical due to the occurrence of anti-drug antibodies (ADAs), more so to infliximab. Individual factors such as genetic background, immune background, weight, and adherence also influence drug response [49] (Mehta and Manson, 2020).

Study limitations

This looks at confronted sure barriers. Time constraints necessitated completion within an exact period, which restricted the potential to gain additional samples, in particular thinking about the predefined treatment period selected to assess primary non-reaction. Additionally, the absence of dedicated investment posed a considerable venture in securing vital sources and expanding the scope of the studies.

Conclusion

The present study highlighted the pivotal role of serum IL-6 levels and the rs1800795 IL-6 gene polymorphism in RA pathogenesis and treatment response. Elevated IL-6 levels were highly associated with raised disease activity and poor therapeutic response to TNF inhibitors. In addition, the C mutant allele at rs1800795 polymorphism was strongly associated with greater disease severity and lower treatment responsiveness, and thus it's useful as a predictive biomarker.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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